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The Composition of the Ceramides from Human Stratum Corneum and from Comedones

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Human epidermal surface lipids were collected by an ethanol wash and the ceramides were quantified by thin-layer chromatography-photodensitometry. Six ceramide fractions were isolated and the structural components of each were analyzed in detail. The most unusual of the epidermal ceramides contained a sphingosine base with amide-linked 30- and 32-carbon ω-hydroxyacids and an ester-linked nonhydroxyacid, 41% of which was linoleic acid. The proportion of linoleic acid in the analogous ceramide from comedones was 6%. This supports the hypothesis that a localized insufficiency of linoleic acid in the follicular epithelium is an etiologic factor in comedogenesis.

Gray and Yardley demonstrated that ceramides accumulate in epidermal cells throughout the differentiation process [1] and represent the major group of lipids in the stratum corneum [1,2]. They also showed that the epidermal ceramides are structurally heterogeneous, but individual structures were not deduced [2,3].

The stratum corneum lipids, including the ceramides, are located in the extracellular spaces where they form multiple broad bilayers lying parallel to the plane of the skin surface [4]. These extracellular lipid structures appear to provide the physical barrier to transepidermal water loss [4–6] and have also been implicated in the cohesive properties of the horny layer [7]. In spite of this apparent functional significance, human epidermal ceramides have not previously been subjected to detailed analysis. However, 7 series of ceramides have been isolated from pig skin and are now structurally defined [8]. In the present report, human ceramides collected by surface extraction with ethanol were compared with the analogous materials from pig epidermis and detailed structures of the human ceramides were determined. In addition, the composition of an octadecadienoic acid-containing ceramide in the skin surface lipids was compared with the corresponding ceramide from comedones.

MATERIALS AND METHODS

Reference Materials

α-Hydroxy fatty acids and ω-hydroxyacids were prepared from wool wax [9] and carnauba wax [10], respectively. Pig epidermal ceramides were prepared as previously described [8]. Sphingosine and phytosphingosine were purchased from Sigma Chemical Company (St. Louis, Missouri) and fatty acid methyl ester standards were from NuCheck Prep (Elysian, Minnesota).

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Abbreviations:
GLC: gas-liquid chromatography
TLC: thin-layer chromatography

Collection of Human Epidermal Lipids

Epidermal lipids were collected from 4 male subjects ranging in age from early twenties through midfifties. All were in generally good health.

Each forearm of the subjects was rinsed with 250 ml of ethanol, and the rinsings were collected in a stainless steel basin. The combined rinsings from both forearms were concentrated via rotary evaporator and taken to dryness under a stream of nitrogen. Final drying of the lipid in a tared culture tube was achieved in vacuo. The tube plus lipid was weighed, and the residue was dissolved in 0.5 ml of chloroform:methanol, 2:1. Four subjects were each sampled 4 times at weekly intervals and the total lipid from each subject was then fractionated into polar and nonpolar components by preparative thin-layer chromatography (TLC). The ceramides were quantitated as described below.

Open and closed comedones were expressed with a Shalita comedone extractor from the face of an otherwise healthy male in his early twenties. After drying in vacuo and weighing, the dried comedones were extracted for 3 successive 2-h intervals with chloroform:methanol (2:1, 1:1, and 1:2). The extracts were combined and processed as described above for the surface extracts.

Thin-layer Chromatography

All TLC employed 20 × 20 cm glass plates coated in the laboratory with a 0.5-mm thickness of silica gel 60H (E. M. Reagents, Darmstadt, West Germany), activated at 110°C and cleaned by development with chloroform:methanol (2:1) prior to use.

For analytical TLC, the adsorbent layer was scored into 6 mm-wide lanes. One sample or standard was applied per lane, and the chromatograms were developed twice with chloroform:methanol:acetic acid (190:9:1) to resolve the ceramides. After development, plates were air dried, sprayed with 50% sulfuric acid, charred, and quantitated by photodensitometry [11,12].

For preparative TLC, each sample was streaked in a narrow band 2 cm from the botton of a plate. To separate polar from nonpolar lipids, the plate was developed with ethyl ether:acetic acid (99:1) and the material remaining below R_f 0.5 was taken as the polar lipids. This area of silica gel was scraped from the plate and transferred to a short glass column. The polar lipids were then eluted with chloroform:methanol:water (50:50:1). To isolate the individual ceramides, the recovered polar lipid fraction was applied to another TLC plate which was then developed twice with chloroform:methanol (19:1). After development, the plate was lightly sprayed with an ethanolic solution of 2′,7′-dichlorofluorescein (Eastman Kodak Co., Rochester, New York). Ceramide-containing regions of silica gel were located under ultraviolet light and scraped from the plate. The ceramides were then eluted with chloroform:methanol (2:1).

Structural Analyses

Each of the human ceramide fractions was subjected to a series of successive chemical and chromatographic analyses essentially as described for analysis of the pig epidermal ceramides [8]. This included: chromatography on sodium arsenite-impregnated silicic acid to detect phytosphingosine-containing structures [8,13]; mild saponification with 1 M KOH in methanol for 1 h at 65°C to detect ester-linked fatty acids; and cleavage of amide linkages by treatment with 1 M HCl in methanol containing 20 M water at 65°C for 18 h [8,14].

Liberated sphingosines were converted to trimethylsilylated derivatives [14] and hydroxyacids were converted to acetylated methyl esters [8] for gas-liquid chromatographic (GLC) analyses, for which a column packed with 3% OV-101 on 80/100 mesh Supelcoport was used [8]. Nonhydroxy fatty acid methyl esters were chromatographed on a 50-m

vitreous silica BP20 capillary column (Scientific Glass Engineering, Inc., Austin, Texas) operated isothermally at 220°C.

RESULTS

The ethanol surface extraction yielded between 31 and 87 mg of total lipid per subject, containing 3–7 mg of polar lipids. A densitometry tracing from a typical TLC analysis of human epidermal ceramides is shown in Fig 1. The average ceramide composition of the samples is presented in Table I, together with the structures determined for the ceramides isolated from the pooled surface lipids. Chain length distributions of the long-chain bases and of the amide-linked acids from the individual epidermal ceramides are presented in Tables II and III, respectively. The ester-linked fatty acid composition of ceramide 1 from the ethanol washes as well as from the comedones is presented in Table IV.

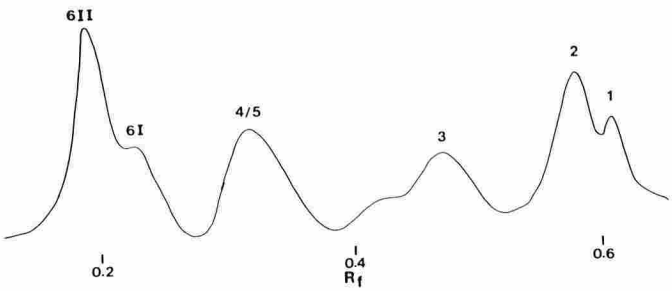

FIG 1. Densitometric profile of a TLC separation of stratum corneum epidermal ceramides.

TABLE I. *Summary of compositional data for human epidermal ceramides*[a]

Ceramide	Weight percent	Long-chain base	Amide-linked acid	Ester-linked acid
1	7.0 ± 3.2[b]	Sphingosine	ω-OH	Non-OH
2	21.0 ± 4.9	Sphingosine	Non-OH	None
3	13.4 ± 4.3	Phytosphingosine	Non-OH	None
4/5	22.2 ± 4.5	Sphingosine	α-OH	None
6I	9.8 ± 1.1	Phytosphingosine	α-OH	α-OH
6II	13.6 ± 4.5	Phytosphingosine	α-OH	None

[a] Ceramides are numbered by analogy with the ceramides from pig epidermis [8]. Weights percent were determined by quantitative thin-layer chromatography. Ceramide fraction 6I also contains an unidentified component.

[b] Standard deviation. N = 4.

TABLE II. *Long-chain bases from human epidermal ceramides*[a]

Chain structure	Sphingosines			Phytosphingosines		
	Cer 1	Cer 2	Cer 4/5	Cer 3	Cer 6I	Cer 6II
16:1	2.1	3.1	1.4	—	—	—
16:0	0.4	3.1	0.4	0.5	1.9	—
17:1	3.3	3.1	3.3	—	—	—
17:0	0.4	2.7	0.7	0.3	2.5	—
18:1	12.6	10.9	31.9	—	—	—
18:0	7.5	14.7	6.5	5.5	16.9	0.6
19:1	3.5	6.6	5.4	—	—	—
19:0	3.0	7.0	5.4	5.5	8.6	3.4
20:1	20.1	3.1	14.5	—	—	—
20:0	3.3	35.3	6.5	18.9	9.4	19.2
21:1	2.1	2.7	5.1	—	—	—
21:0	8.4	tr	7.2	13.8	1.6	15.6
22:1	26.4	tr	5.8	—	—	—
22:0	5.9	7.8	2.5	43.3	34.7	44.5
23:0	—	—	—	2.2	11.3	5.0
24:0	—	—	—	5.2	4.7	6.4
25:0	—	—	—	1.6	1.9	1.8
26:0	—	—	—	2.5	6.6	3.3

[a] Long-chain base composition of each ceramide is presented as weight percent.

TABLE III. *Amide-linked fatty acids from human epidermal ceramides*[a]

Chain structure	ω-Hydroxy fatty acids	Nonhydroxy fatty acids		α-Hydroxy fatty acids		
	Cer 1	Cer 2	Cer 3	Cer 4/5	Cer 6I	Cer 6II
14	—	—	3.1	—	—	—
15	—	—	2.8	—	—	—
16	—	2.0	20.2	11.6	3.0	4.2
17	—	0.4	2.8	1.7	1.0	2.2
18	—	11.4	12.1	4.3	2.3	6.4
19	—	0.4	0.8	0.7	1.0	5.4
20	—	2.8	1.3	3.3	1.0	13.8
21	—	0.5	0.3	0.3	0.3	1.1
22	—	6.9	2.9	2.0	1.5	2.6
23	—	3.0	2.0	2.5	2.1	0.7
24	—	36.5	20.2	31.4	32.8	30.0
25	—	8.0	6.9	6.7	13.4	9.7
26	0.4	21.7	15.0	16.7	34.8	19.9
27	0.2	1.2	1.8	7.2	3.4	5.9
28	4.9	4.0	4.9	4.7	2.1	2.8
29	7.0	0.3	0.7	4.8	0.8	0.9
30	63.6	0.9	1.8	2.0	0.5	1.3
31	8.8	—	—	—	—	—
32	14.9	—	—	—	—	—

[a] Fatty acid compositions are presented as weight percent for each ceramide.

TABLE IV. *Ester-linked fatty acids from ceramide 1*[a]

Chain structure	Stratum corneum	Comedone
14:0	0.5	1.9
15:0	0.4	4.0
16:0	11.7	45.0
16:1	3.2	6.7
17:0	1.4	3.5
17:1	1.5	0.6
18:0	9.1	15.3
18:1	14.6	5.7
18:2	41.3	5.9
19:0	1.0	1.0
19:1	1.5	—
20:0	2.6	2.6
20:1	2.2	—
21:1	1.5	—
22:1	4.8	—
Other	2.7	7.8

[a] Ester-linked fatty acid compositions are presented as weight percent.

The constituents of the human epidermal ceramides, summarized in Table I, were very similar to those recently reported for pig [8]. Human ceramides 1, 2, and 4/5 all contained sphingosine bases ranging from 16–22 carbons in length. In each case, the major species were 18, 20, and 22 carbons long, but as with the pig there were significant levels of some of the odd-carbon chain lengths. Ceramides 3, 6I, and 6II contained phytosphingosines which were mainly 20–22 carbons long. These phytobases were completely saturated, and no methyl branching was detected.

The amide-linked fatty acids, summarized in Table III, ranged from 14–32 carbons long and included nonhydroxy-, α-hydroxy-, and ω-hydroxyacids. Ceramide 1 contained mainly 30 and 32-carbon saturated, straight-chained ω-hydroxyacids. Ceramides 2 and 3 both contained amide-linked nonhydroxy-acids with major amounts of the 18, 24, and 26 carbon entities. In addition, ceramide 3 contained 20% of palmitic acid. Ceramides 4/5, 6I, and 6II all contained α-hydroxyacids in amide linkage. In all 3 cases, the major chain lengths were 24 and 26.

Ceramide 4/5 contained a higher level of α-hydroxypalmitic acid than ceramides 6I and 6II.

In addition to the amide-linked fatty acids, ceramides 1 and 6I contained ester-linked components. In ceramide 6I, the ester-linked α-hydroxyacid had a chain length distribution (data not shown) similar to that of the amide-linked α-hydroxyacid shown in Table III. Ceramide 1 contained ester-linked nonhydroxyacids, the composition of which is presented in Table IV. Octadecadienoic acid was the major ester-linked acid in normal human ceramide 1, but was much reduced in the ceramide 1 sample derived from comedones.

DISCUSSION

The 6 series of human ceramides defined in the present study were structurally quite similar to the ceramides recently isolated from pig epidermis. In both cases, the hydrophobic chains were mainly straight and saturated. These characteristics are in accord with the role proposed for the ceramides in the epidermal barrier in that they would make the intercellular membranes of the stratum corneum both resistant to oxidative damage and relatively impermeable to water [15].

One unusual characteristic of the epidermal ceramides is the extreme range of chain lengths. For instance, the amide-linked fatty acids range from 14–30 carbons in length. Although the major species contain 24 or more carbons, there are also significant amounts of 16 and 18 carbon species, which are more typical of membrane-forming lipids. One implication of this diversity is a greater degree of interdigitation of chains within the hydrophobic portion of the membrane. This greater opportunity for interaction between the two leaflets of the unit membrane may be an important stabilizing factor for the stratum corneum membranes, which do not include phospholipids.

The most unusual of the epidermal ceramides is ceramide 1. This consists of a sphingosine base with an amide-linked long-chain ω-hydroxyacid and an ester-linked nonhydroxyacid, 41% of which proved to be an octadecadienoic acid. This species is probably linoleic acid since it cochromatographed on GLC with authentic octadeca-9,12-dienoic acid and was cleanly resolved from the 5,8-isomer found in human sebum.

It has been proposed that this ceramide ester may serve as a molecular rivet in locking together the multiple intercellular membranes in the stratum corneum [8]. Since ceramide 1 is highly enriched in essential fatty acid and has been shown to survive desquamation [16], it would seem to provide a readily available and sensitive monitor of the essential fatty acid status of the skin.

It has been suggested that a localized deficiency of linoleic acid may be a causative factor in comedogenesis and acne [17]. Our preliminary results presented in Table IV support this hypothesis, since they show that ceramide 1 from comedones contains only one-seventh of the level of linoleate found in ceramide 1 from normal stratum corneum. It must be emphasized that the results presented in Table IV are of a preliminary nature. It would, for instance, have been more meaningful to compare the lipids of comedones with lipids from follicular epithelium; however, it is not presently feasible to collect enough human follicular epithelium to permit such a comparison. Also, it is possible that the ethanol extraction of stratum corneum lipids does not produce a truly representative sample for comparison with the comedonal extract; however, this possibility does not seem likely in view of the favorable comparison between lipids collected by the ethanol wash method and those obtained by extensive chloroform-methanol extraction of exfoliated corneocytes [16]. These problems will be resolved only by much additional work.

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